Supplementary figures

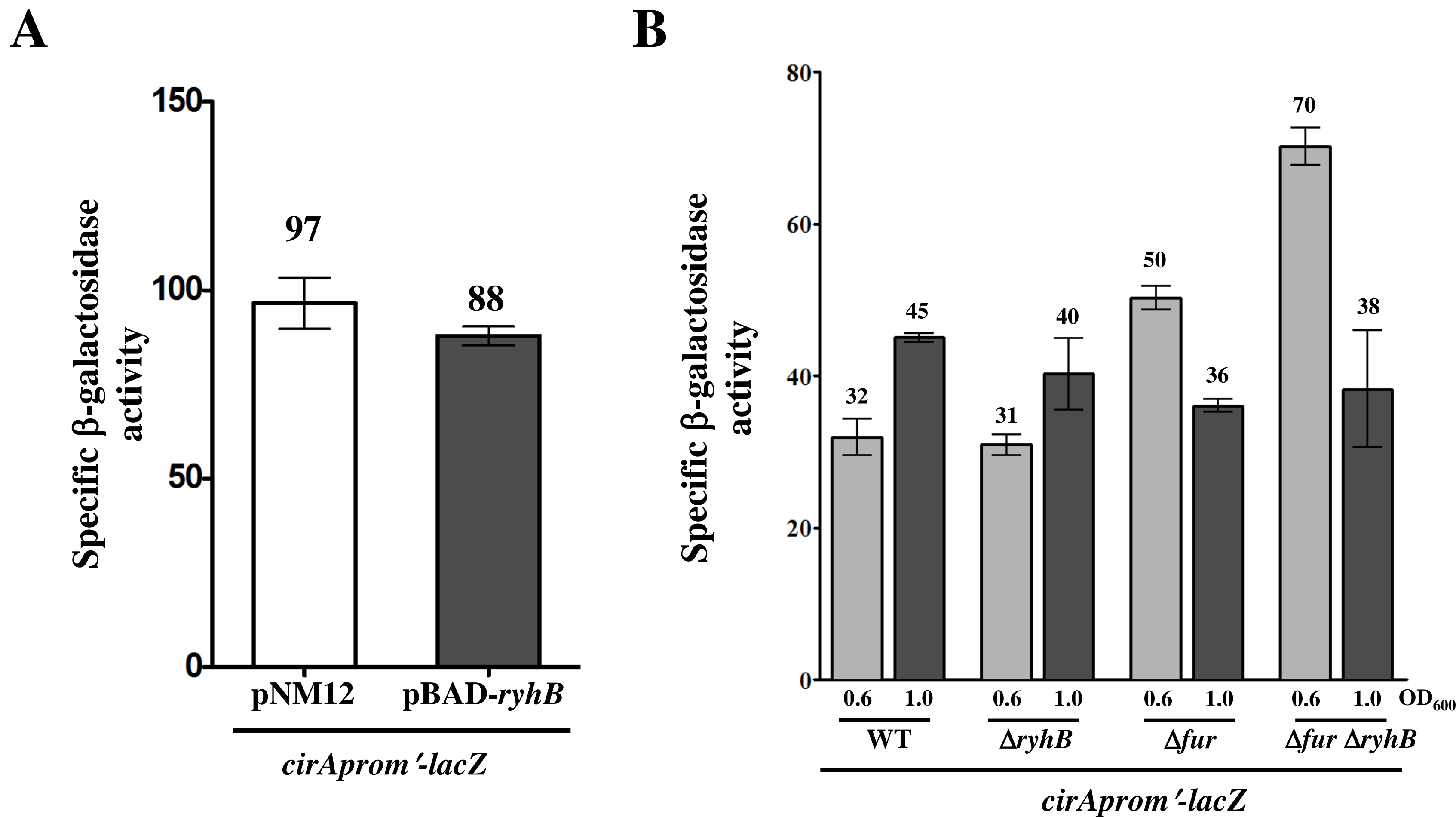


Figure S1. RyhB expression does not affect *cirA* promoter activity. **(A)** Effect of arabinose-induced RyhB on *cirAprom*′*-lacZ* transcriptional fusion in a Δ*fur* Δ*ryhB* background. Cells were grown in LB supplemented with ampicillin and 0.1% arabinose was added to an OD_{600} of 0.1. Specific β-galactosidase activity from three independent cultures was then measured 3 hours later. Mean and standard deviation (SD) values are shown. The empty vector pNM12 was used as a control. **(B)** β-galactosidase assay of *cirAprom*′*-lacZ* transcriptional fusion in WT, Δ*ryhB*, Δ*fur*, Δ*fur* Δ*ryhB* cells grown in M63 iron-free glucose minimal medium at the indicated OD_{600}. Specific β-galactosidase activity from three independent cultures was measured. Mean and SD values are shown.

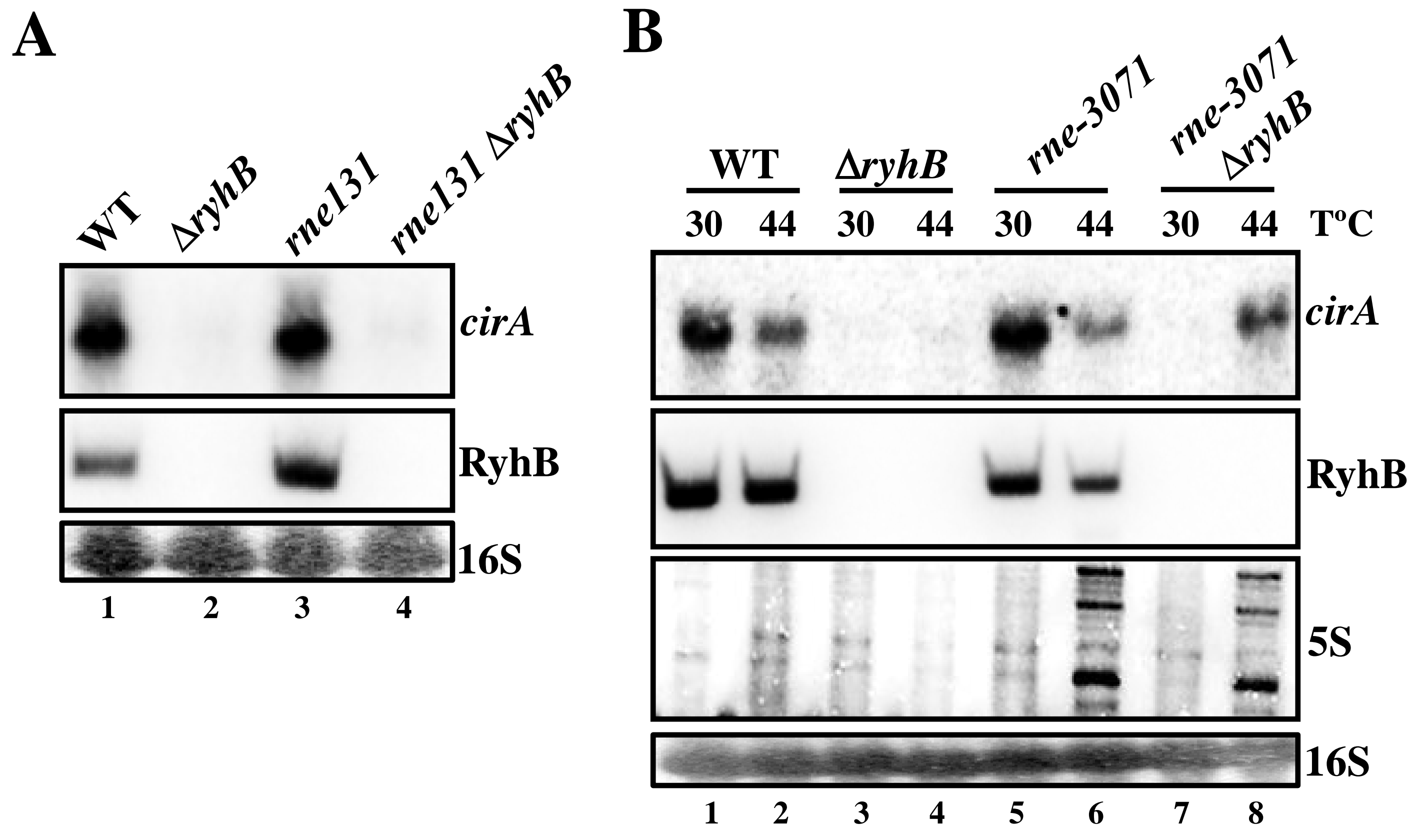


Fig S2. RyhB protects *cirA* mRNA from RNase E degradation. **(A)** Northern blot analysis of *rne131* mutation effect on *cirA* mRNA levels. Strains EM1055 (WT), EM1238 (D*ryhB*), EM1377 (*rne131*) and JF133 (*rne131* D*ryhB*) were grown in M63 iron-free glucose minimal medium and total RNA was extracted at an OD_{600} of 0.6. A probe complementary to *cirA* open reading frame (ORF) was used. **(B)** Northern blot analysis of RNase E inactivation effect on *cirA* mRNA levels. Strains EM1055 (WT), EM1238 (D*ryhB*), EM1277 (*rne-3071*) and EM1280 (*rne-3071* D*ryhB*) were grown at 30°C (permissive temperature) in M63 iron-free glucose minimal medium at an OD_{600} of 1.0. At this point, cultures were transferred at 44°C (restrictive temperature) and total RNA was extracted 15 min later. A probe complementary to *cirA* ORF was used. Note the accumulation of unprocessed 5S rRNA intermediates, consistent with the inactivation of RNase E (Apirion and Lassar, 1978).

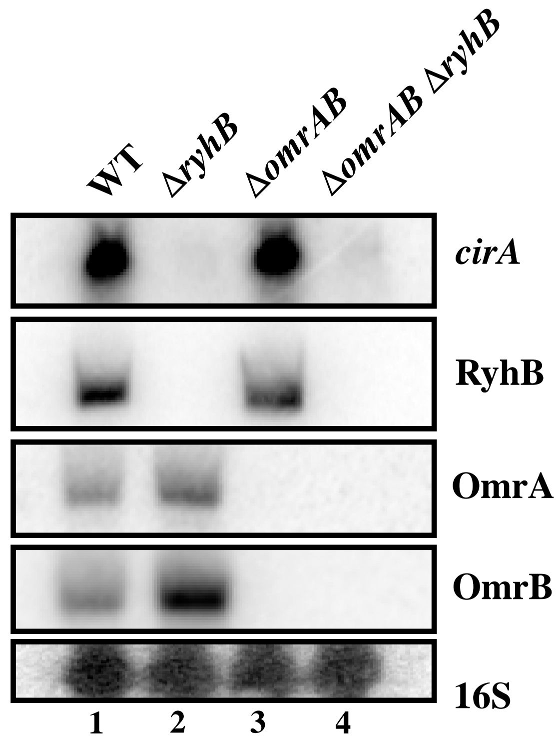


Figure S3. *omrAB* inactivation in Δ*ryhB* background do not result in increased levels of *cirA* mRNA. Northern blot analysis of OmrA and OmrB effect on *cirA* mRNA levels. Strains EM1055 (WT), EM1238 (Δ*ryhB*), HS364 (Δ*omrAB*) and HS365 (Δ*omrAB* Δ*ryhB*) were grown in M63 iron-free glucose minimal medium at an OD_{600} of 0.6 and total RNA was extracted.

Figure S4. (A). Toeprint analysis of Hfq effect on 30S ribosomal subunit binding to *lpp* mRNA upon translation initiation. Unlabeled *lpp* mRNA was incubated in the presence of the indicated amounts of purified Hfq protein. Positions +15/+16 relative to *lpp* AUG start codon are indicated. CTAG refers to sequencing ladders generated with the same primers used for toeprint (EM2174 for *lpp*). Toeprint signals were quantified by densitometry and data are reported in arbitrary units. **(B).** Toeprint analysis of RyhB effect on 30S ribosomal subunit binding to *lpp* mRNA in the presence of Hfq upon translation initiation. Unlabeled *lpp* mRNA (0.2 µM final) was incubated in the presence of Hfq (0.15 µM) and RyhB (0.15 µM).

A

5′ ... 3′

SL1

SL2

SL3

Hfq I (-105 to -91)

Hfq II (-33 to -31)

Hfq III (+4 to +6)

START

SD

RyhB

A → C(ML)

RyhB pairing core (-51 to -42)

B

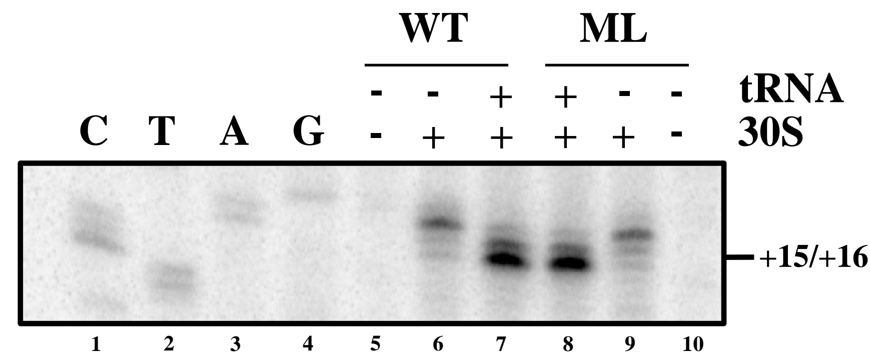


Figure S5. Toeprint analysis of 30S binding to *cirA*ML mRNA upon translation initiation. **(A)** RNAfold prediction of *cirA* 5′-UTR secondary structure (nucleotides -173 to +18 relative to the AUG start codon). ML mutation is indicated. **(B)** Unlabeled *cirA* and *cirA*ML mRNAs were incubated in the presence of 30S ribosomal unit and tRNA^{fmet}. Positions +15/+16 relative to *cirA* AUG start codon are indicated. CTAG refers to sequencing ladders generated with the same primer used for toeprint (EM1408).

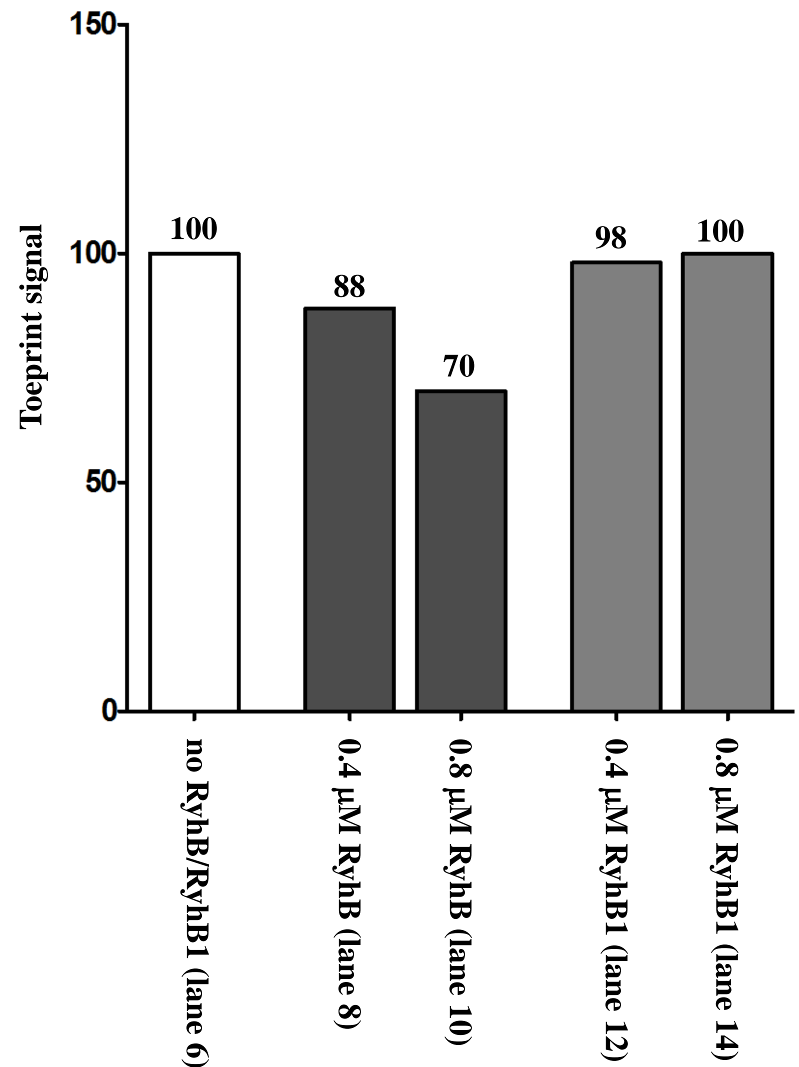
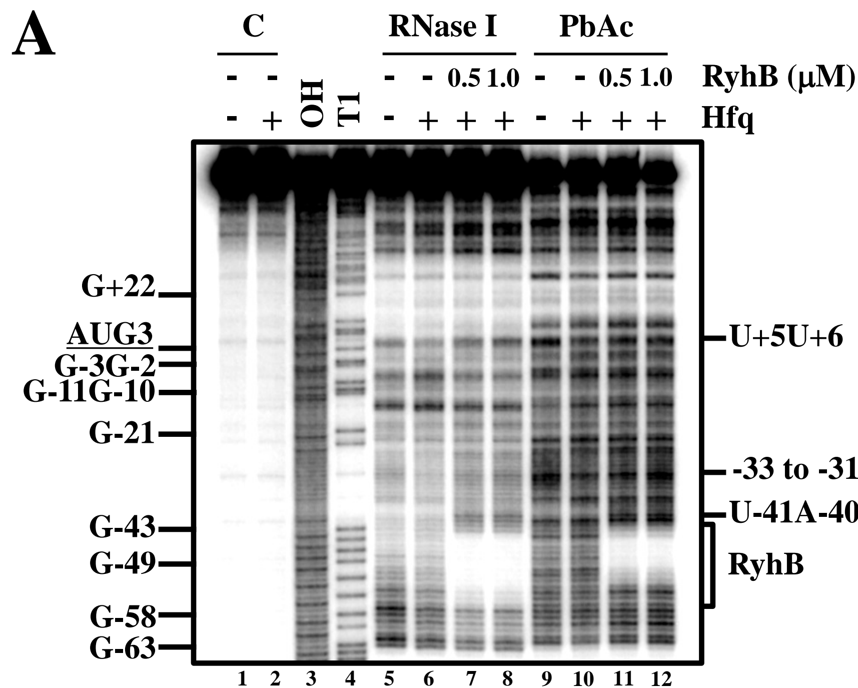
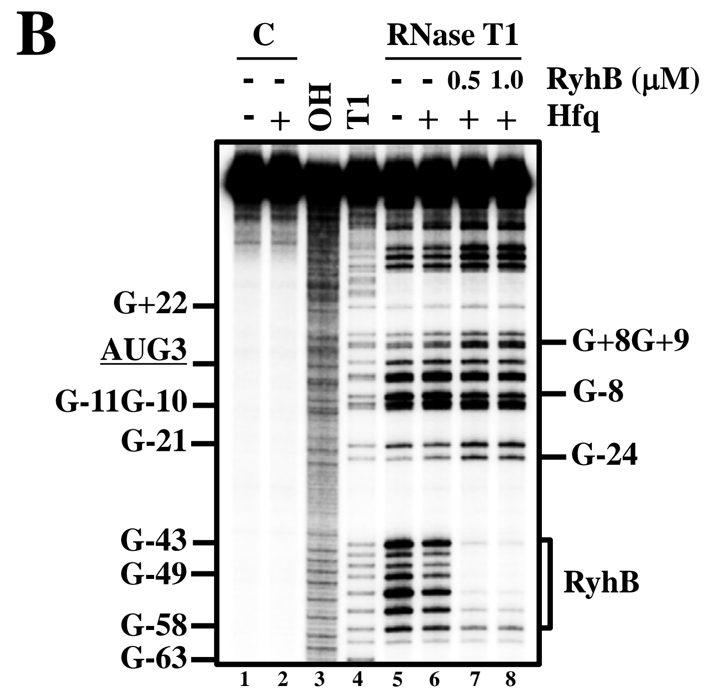


Figure S6. Toeprint analysis of RyhB effect on 30S binding to *cirA* mRNA upon translation initiation. Unlabeled *cirA* mRNA was incubated in the presence of the indicated concentrations of RyhB and RyhB1. Positions +15/+16 relative to *cirA* AUG start codon are indicated. Formation of RyhB-*cirA* RNA duplex results in a reverse transcription block at position G-45. CTAG refers to sequencing ladders generated with the same primer used for toeprint (EM1408). Toeprint signals were quantified by densitometry.





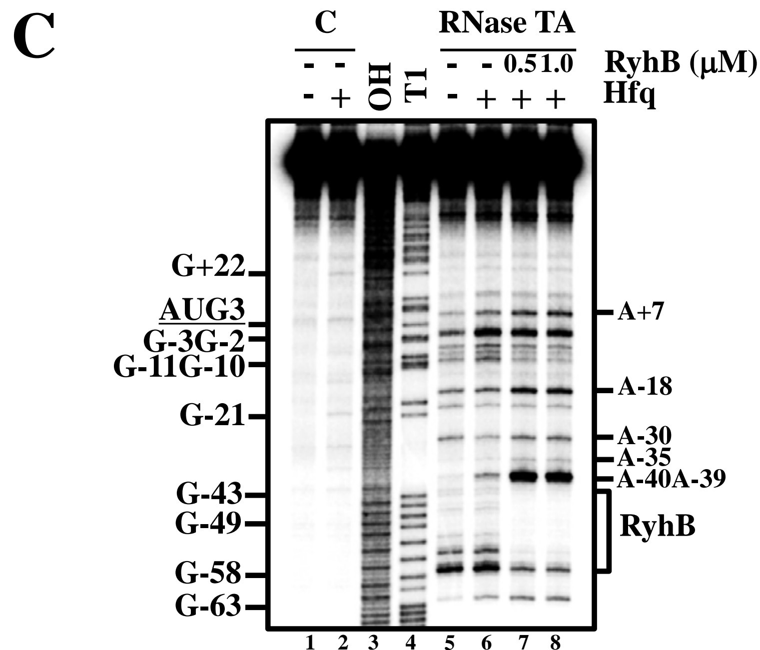


Figure S7. Enzymatic and chemical *in vitro* probing of Hfq and RyhB effects on *cirA* mRNA. 5′-end-labeled *cirA* mRNA (nucleotides -173 to +78 relative to the AUG start codon) was incubated in the presence 0.3 μM Hfq and the indicated amounts of RyhB before the addition of either **(A)** PbAc and RNase I **(B)** RNase T1 or **(C)** RNase TA. Some guanine positions are given for orientation. (C) Nonreacted controls; (OH) alkaline ladder; (T1) RNase T1 ladder (guanine residues).

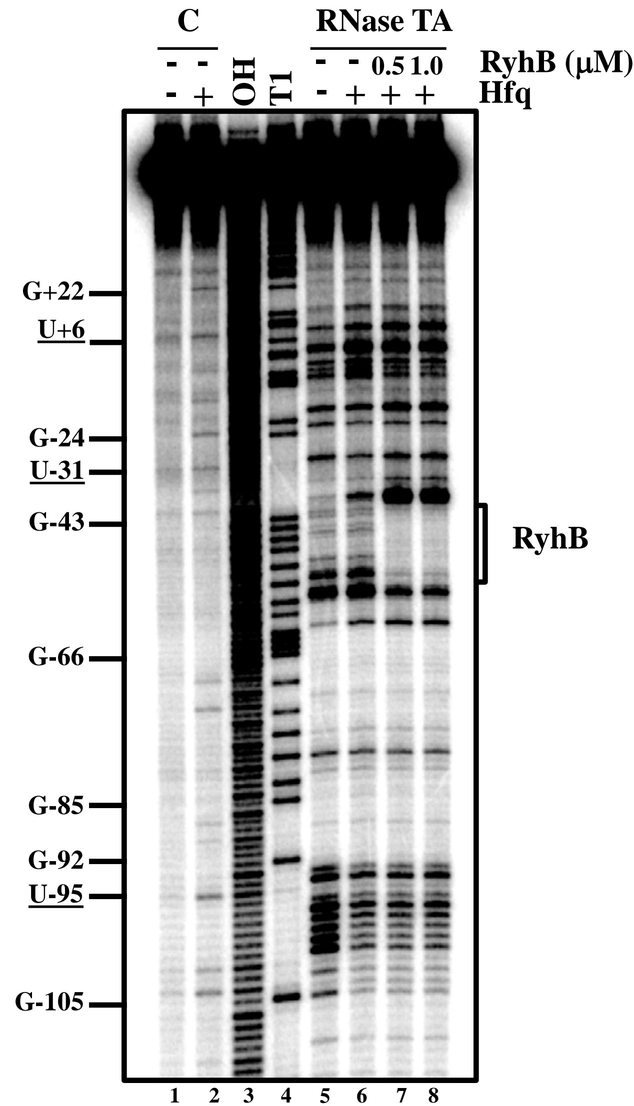


Figure S8. Hfq preparation carries a residual RNase activity. 5′-end-labeled *cirA* mRNA (nucleotides -173 to +78 relative to the AUG start codon) was incubated 30 minutes in the presence of Hfq. Some nucleotides for which cleavage induction upon Hfq addition was observed are underlined (see control samples). Guanine (C) Nonreacted controls; (OH) alkaline ladder; (T1) RNase T1 ladder (guanine residues).

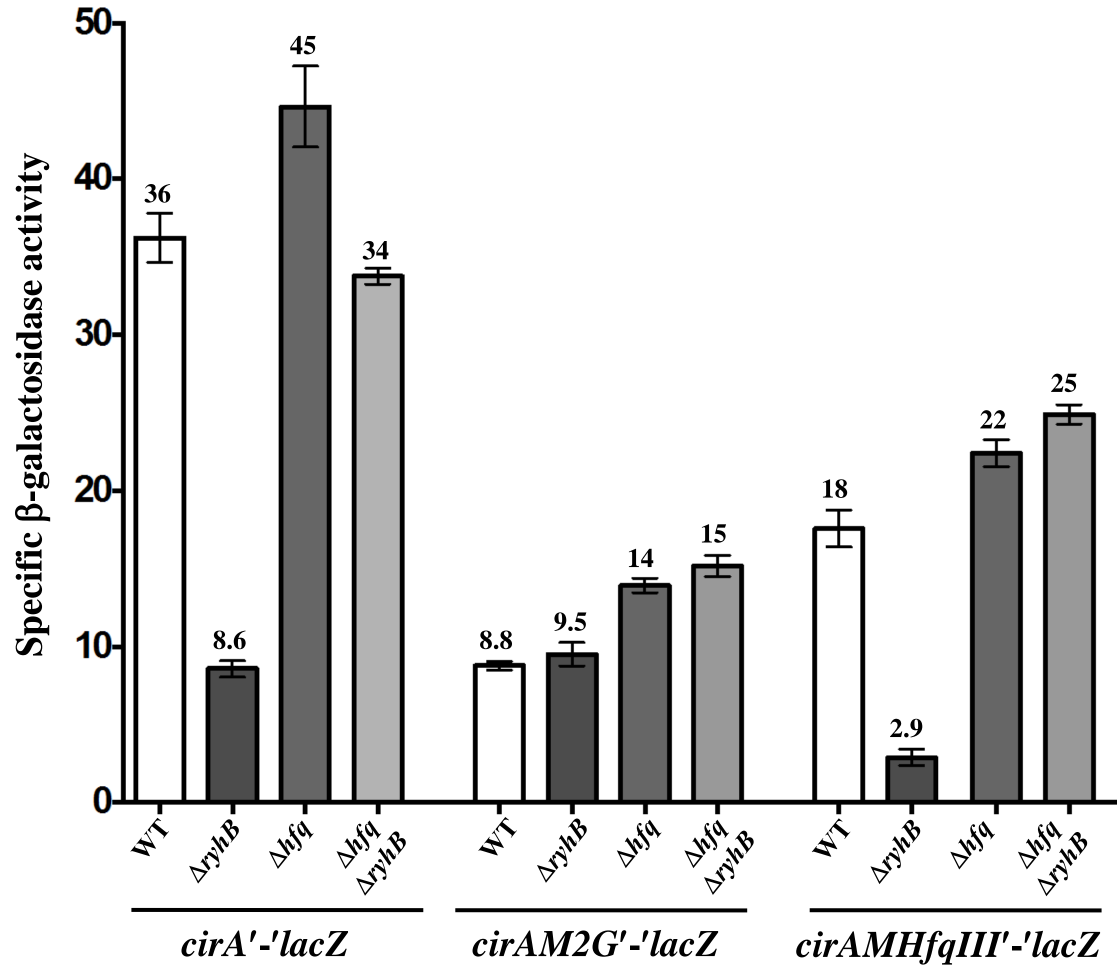


Figure S9. β-galactosidase assay of *cirA'-'lacZ*, *cirAM2G'-'lacZ*, and *cirAMHfqIII'-'lacZ* translational fusions in Δ*hfq* and Δ*hfq* Δ*ryhB* cells grown in M63 iron-free glucose minimal medium at an OD_{600} of 0.6. Specific β-galactosidase activity from three independent cultures was measured. Mean and SD values are shown.

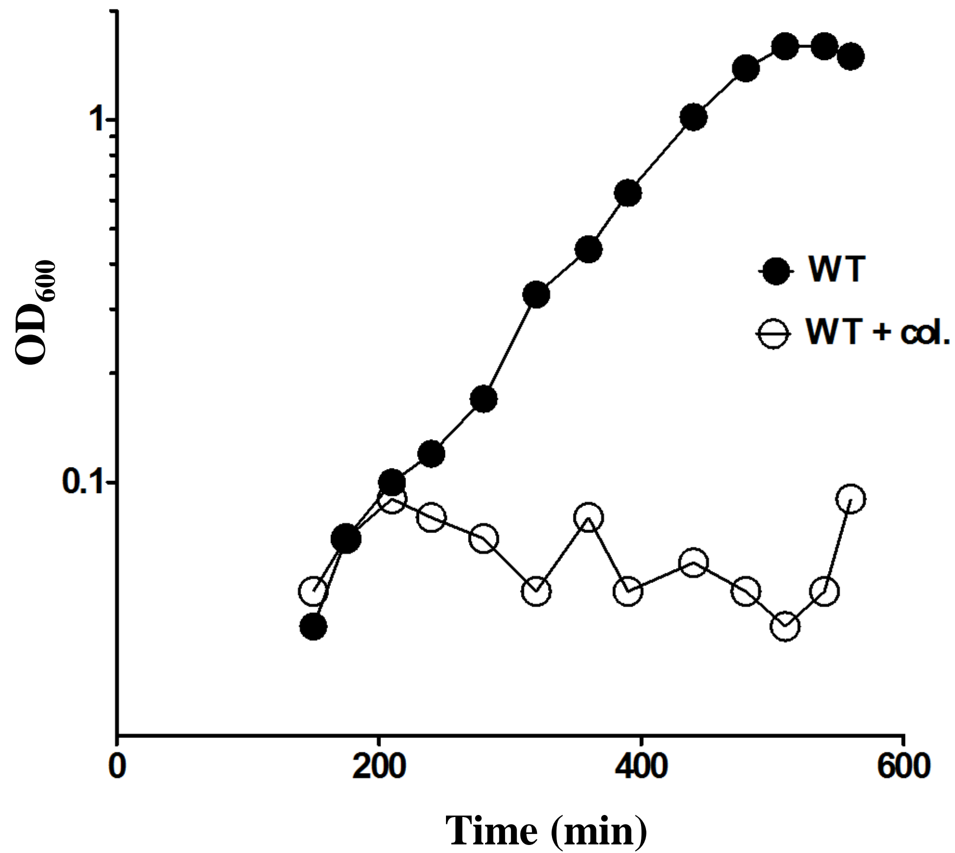


Figure S10. Colicin-treated WT cells do not develop resistance to colicin Ia over 24 hours. WT cells previously treated with colicin Ia for 24 hours were rediluted in fresh iron-free minimal medium and treated again with colicin Ia.

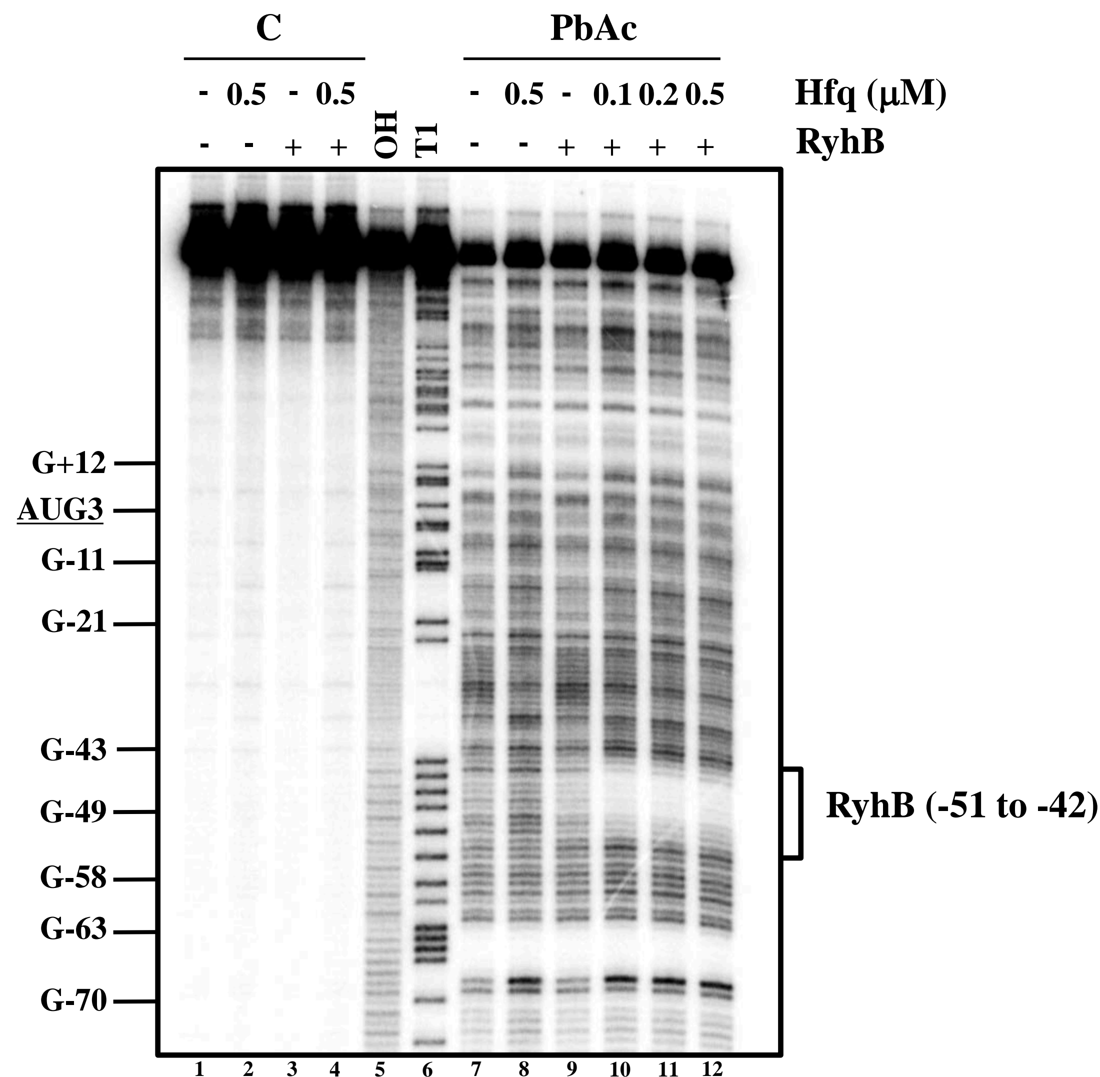
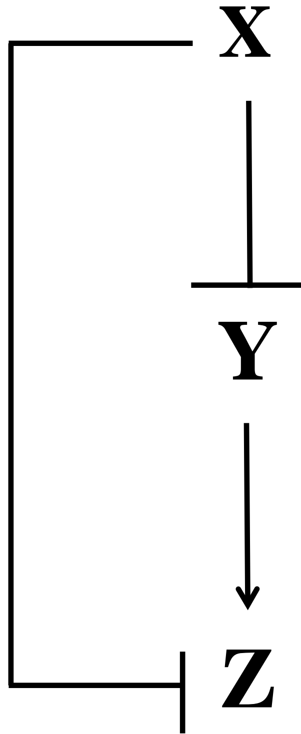


Figure S11. Lead acetate (PbAc) probing of Hfq effect on RyhB pairing with *cirA* mRNA. 5′-end-labeled *cirA* mRNA (nucleotides -173 to +78 relative to the AUG start codon) was incubated in the presence of 0.2 µM RyhB and of the indicated amounts of purified Hfq protein before the addition of PbAc. Some guanine positions are given for orientation. (C) Nonreacted controls; (OH) alkaline ladder; (T1) RNase T1 ladder (guanine residues).

A

Coherent type 2 feed-forward loop



B

Fur-RyhB-*cirA* regulation

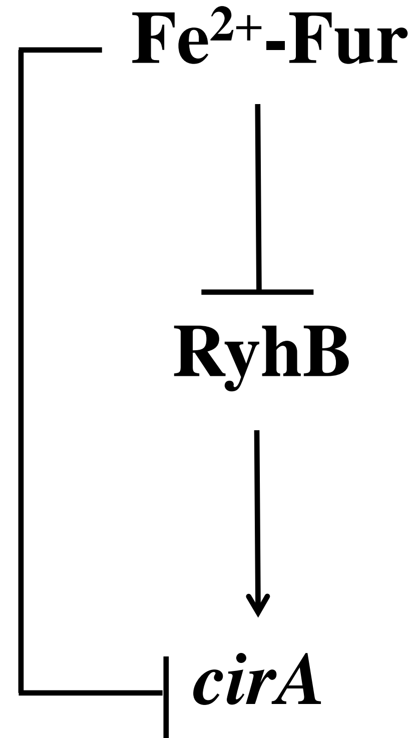


Figure S12. Fur-RyhB-*cirA* forms a coherent type 2 feed-forward loop. **(A)** Structure of a coherent type 2 feed-forward loop of a transcription network in which transcription factor X represses promoter Z and transcription factor Y which activates promoter Z. Adapted from Mangan and Alon, 2003. **(B)** Fur-RyhB-*cirA* coherent type 2 feed-forward loop in which Fe^{2+}-Fur corresponds to X, RyhB to Y and *cirA* promoter to Z.

Supplementary Materials and Methods

Construction of B-galactosidase fusions. For the construction of *cirA′-lacZ* transcriptional and *cirA′-′lacZ* translational fusions, a PCR fragment containing -280 to +32 relative to the *cirA* start codon (oligos EM1361-EM1365) was digested by *Eco*RI and *Bam*HI and ligated into *Eco*RI/*Bam*HI-digested pFRΔ (Repoila and Gottesman, 2001) and pRS1551 (Simons *et al.*, 1987) to generate transcriptional and translational fusions, respectively. For the construction of *cirAprom′-lacZ* transcriptional fusion, a PCR fragment containing -280 to -149 relative to the *cirA* start codon (oligos EM1361-EM1454) was digested by EcoRI and BamHI and ligated into *Eco*RI/*Bam*HI-digested pFRΔ. For *cirAmutAUG′-lacZ* transcriptional fusion, a PCR fragment containing -280 to +32 relative to the mutated start codon was generated (EM1361-EM1680) and ligated into *Eco*RI/*Bam*HI-digested pFRΔ. To generate *cirA1′-′lacZ* fusion, two independent PCR reactions (oligos EM1361-EM1403 and EM1365-EM1402) were performed. The two PCR products were then mixed to serve as the template for a third PCR (oligos EM1361-EM1365). The resulting PCR product was then digested by *Eco*RI and *Bam*HI and ligated into *Eco*RI/*Bam*HI-digested pRS1551. To generate *cirAM2G′-′lacZ* and *cirAMHfqIII′-′lacZ* translational fusions, PCR reactions using EM1361-EM2170 and EM1361-EM2171, respectively, were performed. The resulting PCR products were then digested by *Eco*RI and *Bam*HI and ligated into *Eco*RI/*Bam*HI-digested pRS1551. The transcriptional and translational fusions were delivered in single copy into the bacterial chromosome at the λ *att* site as described previously (Simons *et al.*, 1987). Stable lysogens were screened for single insertion of recombinant λ by PCR (Powell *et al.*, 1994). To construct pBAD-*ryhB1*, the original vector pBAD-*ryhB* (Massé *et al*, 2003) was used as a template for two PCR reactions (oligos EM168-EM1446 and EM1445-EM169). The two products were mixed to serve as the template for a third PCR reaction (oligos EM168-EM169). The resulting PCR product was digested by *Msc*I and *Eco*RI and inserted into *Msc*I/*Eco*RI-digested pNM12 (Majdalani *et al,* 1998).

In vitro RNA synthesis and radiolabeling. The radiolabeled probes used for Northern blot analysis and the RNAs used for secondary structure probing and toeprinting were transcribed from a PCR product using a purified T7 RNA polymerase. Transcription was performed in T7 transcription buffer (80 mM HEPES-KOH pH 7.5, 24 mM $MgCl_2$, 40 mM DTT, 2 mM spermidine), 400 μM NTPs (A, C, and G), 10 μM UTP, 3 μl of α-^{32}P-UTP (3000 Ci/mmol), 20 U of RNase OUT (Invitrogen), 5 μg of T7 RNA polymerase, and 1 μg of DNA template. After 4 h of incubation at 37°C, the mixture was treated with 2U of TURBO™ DNase (Ambion), extracted once with phenol-chloroform and purified on denaturing acrylamide gel for transcripts used for secondary structure probing and toeprinting, and on G-50 Sephadex column for radiolabeled probes. For 5′-end labeling, transcripts were dephosphorylated with calf intestine phosphatase (New England Biolabs) and 5′-end-labeled with [^{32}P]-γ-ATP using T4 polynucleotide kinase (New England Biolabs), according to the manufacturer's protocol. Radiolabeled transcripts were purified on denaturing acrylamide gels before use. The oligos used for generating DNA templates for radiolabelled probes synthesis were EM1456-EM1457 (*cirA*), EM253-EM254 (*shiA*), EM190-EM191 (RyhB), EM188-EM189 (*sodB*), EM1771-EM1772 (*yncE*), EM293-EM294 (16S) and EM192-EM193 (5S). For the transcripts used for

secondary structure probing and toeprinting, EM1092-EM1093 (*cirA*), EM2173-EM2174 (*lpp*), EM2065-EM2066 (*yncE*) and EM88-EM89 (RyhB) were used to generate DNA templates. For the construction of *cirA1*, *cirA*M2G and *cirA*ML DNA templates, two independent PCR reactions (EM1092-EM1403 and EM1402-EM1093 for *cirA1*; EM1092-EM2051 and EM2050-EM1093 for *cirA*M2G; EM1092-EM2025 and EM2024-EM1093 for *cirA*ML) were performed. The two PCR products were then mixed to serve as a template for a third PCR (EM1092-EM1093). For the construction of RyhB1 DNA template, a PCR reaction with oligos EM88-EM89 was performed by using pBAD-*ryhB1* plasmid as template. For DNA radiolabeled probes, the oligos EM1583 (OmrA) and EM1584 (OmrB) were directly 5′-end labeled and purified on G-50 Sephadex column.

Colicin sensitivity tests. Colicin Ia was extracted from a strain harbouring p3Z/ColIa plasmid as described earlier (Brickman and Armstrong, 1996). For colicin sensitivity tests, overnight cultures of EM1055, EM1238 and HS221 were diluted in fresh iron-free M63, 0.2% glucose minimal medium and were grown at 37°C. A 1:15000 dilution of cleared colicin lysate or 50 mM HEPES buffer pH 7.4 (untreated cultures) was then added at an OD_{600} of 0.1. When needed, 33 μM of 2,3-dihydroxybenzoic acid (DHB) or ethanol (untreated cultures) was added 30 min before colicin addition.

Table 1. Strains used in this study

Strain	Relevant markers	Reference/Source
DM1187	*lexA51 lexA3*	Mount (1977)
EM1053	SG1039 [Δ*lac proC* mutant *zaj-403*::*tet*]	Susan Gottesman
EM1055	MG1655 Δ*lac* X174	Massé and Gottesman (2002)
EM1237	DY330 [W3110 Δ*lacU169 gal490* λ*cI857* Δ(*cro-bioA*)]	Yu *et al* (2000)
EM1238	EM1055 Δ*ryhB*::*cat*	Massé and Gottesman (2002)
EM1256	EM1055 Δ*fur*::*kan*	Massé and Gottesman (2002)
EM1265	EM1055 *hfq-1*::Ω(*kan;Bcl1*)	Massé *et al* (2003)
EM1277	EM1055 *rne-3071 zce-726*_Tn10	Massé *et al* (2003)
EM1280	EM1055 *rne-3071 zce-726*_Tn10 Δ*ryhB*::*cat*	Massé *et al* (2003)
EM1377	EM1055 *rne-131 zce-726*_Tn10	Massé *et al* (2003)
EM1451	EM1055 Δ*ara714 leu*$^{+}$	Desnoyers *et al* (2009)
EM1455	EM1055 Δ*ara714 leu*$^{+}$ Δ*ryhB*::*cat*	Desnoyers *et al* (2009)
EM1480	EM1055 Δ*ara714 leu*$^{+}$ Δ*fur*::*kan*	EM1451 + P1(EM1256)
EM1493	EM1055 Δ*ara714 leu*$^{+}$ Δ*fur*::*kan* Δ*ryhB*::*cat*	EM1480 + P1(EM1238)
GD520	EM1055 Δ*araB::kan*	This study
HS221	EM1055 Δ*cirA::tet*	This study
HS324	EM1055 λ[*cirAprom'-lacZ*]	EM1055 + λ[*cirAprom'-lacZ*]
HS325	EM1055 Δ*ryhB*::*cat* λ[*cirAprom'-lacZ*]	EM1238 + λ[*cirAprom'-lacZ*]
HS326	EM1055 Δ*fur*::*kan* λ[*cirAprom'-lacZ*]	KP392 + λ[*cirAprom'-lacZ*]
HS327	EM1055 Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirAprom'-lacZ*]	KP393 + λ[*cirAprom'-lacZ*]
HS364	EM1055 Δ*omrAB::tet*	This study
HS365	EM1055 Δ*ryhB*::*cat* Δ*omrAB::tet*	EM1238 + P1(HS364)
HS397	EM1055 Δ*araB*-scar	This study
HS399	EM1055 Δ*ara714 leu*$^{+}$ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirAmutAUG'-lacZ*]	EM1493 + λ[*cirAmutAUG'-lacZ*]
HS400	EM1055 λ[*cirAmutAUG'-lacZ*]	EM1055 + λ[*cirAmutAUG'-lacZ*]

HS401	EM1055 Δ*ryhB*::*cat* λ[*cirAmutAUG′-lacZ*]	EM1238 + λ[*cirAmutAUG′-lacZ*]
HS437	EM1055 Δ*cirA::tet* Δ*fur*::*kan*	EM1055 + P1(EM1256)
HS480	EM1055 λ[*cirA′-lacZ*] Δ*hfq-722::kan*	JUB015 + P1(JW4130-1)
HS481	EM1055 Δ*ryhB*::*cat* λ[*cirA′-lacZ*] Δ*hfq-722::kan*	JUB016 + P1(JW4130-1)
HS482	EM1055 λ[*cirA′- ′lacZ*] Δ*hfq-722::kan*	JUB019 + P1(JW4130-1)
HS483	EM1055 λ[*cirA′- ′lacZ*] Δ*ryhB*::*cat* Δ*hfq-722::kan*	JUB020 + P1(JW4130-1)
HS503	EM1055 Δ*fur::tet*	This study
HS504	EM1055 Δ*araB*-scar Δ*ryhB*::*cat*	HS397 + P1(EM1238)
HS506	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet*	HS504 + P1(HS503)
HS518	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet* Δ*hfq-722::kan*	HS506 + P1(JW4130-1)
HS519	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet* λ[*cirA′-lacZ*]	HS506 + λ[*cirA′-lacZ*]
HS520	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet* λ[*cirA′- ′lacZ*]	HS506 + λ[*cirA′- ′lacZ*]
HS527	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet* λ[*cirA′-lacZ*] Δ*hfq-722::kan*	HS519 + P1(JW4130-1)
HS528	EM1055 Δ*araB*-scar Δ*ryhB*::*cat* Δ*fur::tet* λ[*cirA′- ′lacZ*] Δ*hfq-722::kan*	HS520 + P1(JW4130-1)
JF133	EM1055 *rne-131 zce-726*_Tn10 Δ*ryhB*::*cat*	EM1377 + P1(EM1238)
JW4130-1	*rrnB3* Δ*lacZ4787 hsdR514* Δ(*araBAD*)567 Δ(*rhaBAD*)568 *rph-1* Δ*hfq-722*::*kan*	Baba *et al* (2006)
JUB004	EM1055 Δ*ara714* leu^+ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirA′- ′lacZ*]	EM1493 + λ[*cirA′- ′lacZ*]
JUB006	EM1055 Δ*ara714* leu^+ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirA′-lacZ*]	EM1493 + λ[*cirA′-lacZ*]
JUB013	EM1055 Δ*ara714* leu^+ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirA′-lacZ*]	JUB006 + pNM12
JUB014	EM1055 Δ*ara714* leu^+ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirA′-lacZ*]	JUB006 + pBAD-*ryhB*
JUB015	EM1055 λ[*cirA′-lacZ*]	EM1055 + λ[*cirA′-lacZ*]
JUB016	EM1055 Δ*ryhB*::*cat* λ[*cirA′-lacZ*]	EM1238 + λ[*cirA′-lacZ*]

JUB019	EM1055 λ[*cirA′-′lacZ*]	EM1055 + λ[*cirA′-′lacZ*]
JUB020	EM1055 Δ*ryhB*::*cat* λ[*cirA′-′lacZ*]	EM1238 + λ[*cirA′-′lacZ*]
JUB026	EM1055 Δ*ara714 leu*$^{+}$ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirA1′-′lacZ*]	EM1493 + λ[*cirA1′-′lacZ*]
JUB043	EM1055 Δ*ara714 leu*$^{+}$ Δ*fur*::*kan* Δ*ryhB*::*cat* λ[*cirAprom′-lacZ*]	EM1493 + λ[*cirAprom′-lacZ*]
KP111	EM1055 *hfq-1*::Ω(*kan;Bcl1*) Δ*ryhB*::*cat*	EM1265 + P1(EM1238)
KP392	EM1055 Δ*fur*::*kan*	Salvail *et al* (2010)
KP393	EM1055 Δ*fur*::*kan* Δ*ryhB*::*cat*	Salvail *et al* (2010)
MPC252	EM1055 Δ*fur::tet* Δ*ryhB*::*cat*	HS503 + P1(EM1238)
MPC253	EM1055 Δ*fur::tet* Δ*ryhB*::*cat* Δ*hfq-722::kan*	MPC252 + P1(JW4130-1)
MPC257	EM1055 λ[*cirAM2G′-′lacZ*]	EM1055 + λ[*cirAM2G′-′lacZ*]
MPC258	EM1055 λ[*cirAM2G′-′lacZ*] Δ*ryhB*::*cat*	MPC257 + P1(EM1238)
MPC259	EM1055 λ[*cirAMHfqIII′-′lacZ*]	EM1055 + λ[*cirAMHfqIII′-′lacZ*]
MPC260	EM1055 λ[*cirAMHfqIII′-′lacZ*] Δ*ryhB*::*cat*	MPC259 + P1(EM1238)
MPC263	EM1055 λ[*cirAM2G′-′lacZ*] Δ*hfq-722::kan*	MPC257 + P1(JW4130-1)
MPC264	EM1055 λ[*cirAM2G′-′lacZ*] Δ*hfq-722::kan* Δ*ryhB*::*cat*	MPC263 + P1(EM1238)
MPC267	EM1055 λ[*cirAMHfqIII′-′lacZ*] Δ*hfq-722::kan*	MPC259 + P1(JW4130-1)
MPC268	EM1055 λ[*cirAMHfqIII′-′lacZ*] Δ*hfq-722::kan* Δ*ryhB*::*cat*	MPC267 + P1(EM1238)

Table 2. Plasmids used in this study

Plasmid	Description	Reference/Source
pNM12	pBAD24 derivative	Majdalani *et al* (1998)
pBAD-*ryhB*	pBAD24 + *ryhB* (arabinose inducible promoter)	Massé *et al* (2003)
pBAD-*ryhB1*	pBAD24 + *ryhB1*	This study
pFRΔ	pRS1553 derivative (for transcriptional fusions).	Repoila and Gottesman (2001)
pRS1551	Plasmid for construction of translational fusions.	Simons *et al* (1987)
pCP20	FLP recombinase expression.	Cherepanov and Wackernagel (1995)
pKD4	Template plasmid for kanamycin resistance.	Datsenko and Wanner (2000)
p3Z/ColIa	High-copy-number plasmid allowing constitutive high-level of colicin Ia expression.	Brickman and Armstrong (1996)

Table 3. Oligonucleotides used in this study

Oligo number	Sequence 5′-3′
EM88	TGTAATACGACTCACTATAGGGCGATCAGGAAGACCCTCGC
EM89	AAAAGCCAGCACCCGGCTGGC
EM168	TCACACTTTGCTATGCCATAGC
EM169	CTGCAGGTCGACTCTAGAGG
EM188	TAATACGACTCACTATAGGGAGACCAGGCAGTTCCAGTAGAAAG
EM189	GCTAAAGATGCTCTGGCACCG
EM190	TAATACGACTCACTATAGGGAGACAGCACCCGGCTGGCTAAG
EM191	CGATCAGGAAGACCCTCGC
EM192	TAATACGACTCACTATAGGGAGATGCCTGGCAGTTCCCTACTC
EM193	TGCCTGGCGGCAGTAGCG
EM215	ACTCGACATCTTGGTTACCG
EM216	CAAGAGGGTCATTATATTTCG
EM217	AAAGCCACGTTGTGTCTCAA
EM218	GCGCTGAGGTCTGCCTCGTG
EM253	TAATACGACTCACTATAGGGAGAGTCCCAGTCGGTCGCCAAAG
EM254	TCTCCACTCGTCCCGATGAAG
EM293	TAATACGACTCACTATAGGGAGACGCTTTACGCCCAGTAATTCC
EM294	CTCCTACGGGAGGCAGCAGT
EM345	TCCGCCACTCGTCAGCAAAGAA
EM392	GGAACCGTGAGACAGGTGCT
EM393	ACCGCTGGCAACAAAGGATA
EM884	GTCGGTTATCAGGTTGCCAGTG
EM885	GAAATCAGGCATCCAGCCAGC
EM981	TATTCAGGGCGTGGAAACCGAACT

EM982	AGCGTACCGTTAGCAGTATGGAAC
EM1044	ATCGTTACGCCGCAATCAAAAAAGGCTGACAAATCAGAGGACTCGACATCTTGGTTACCG
EM1045	CTGATACGATCTTTCACATCGTTACGGAAAACGGTAACGCTGCAAGAGGGTCATTATATTTCG
EM1092	TGTAATACGACTCACTATAGGATCGTTACGCCGCAATCAAAA
EM1093	GACCGCTAACACAGGCCATGC
EM1361	CAGTTGAATTCCTTGCTAAGCCCTCTCAACCG
EM1365	GCATCGGATCCCCGACCCGTACGAAAGGGTTCA
EM1388	CTGTTTCTCCATACCCGTTTTTTTGGATGGAGTGAAACGGTGTAGGCTGGAGCTGCTTC
EM1389	CGCCTGCGCCAACTGCGCCCATATGGCAGTCAAACGCGCCCATATGAATATCCTCCTTAG
EM1402	GTGAAGAAGATGTCAGCGATAAC
EM1403	GTTATCGCTGACATCTTCTTCAC
EM1408	CCATGCACAAGAAATAGCGGAC
EM1445	GCACGACATTGCTGACATTGCTTC
EM1446	GAAGCAATGTCAGCAATGTCGTGC
EM1454	GCATCGGATCCCCTTTTTTGATTGCGGCGTAAC
EM1456	TAATACGACTCACTATAGGGAGAACCGCATCGCTAAGTTTGTCGT
EM1457	GCGAACTGAAATACTACGGTG
EM1583	GAGACAGGGTACGAAGAGCGTACCGAATAATCTCACC
EM1584	GTGTAATTCATGTGCTCAACCCGAAGTTGACTTCACC
EM1599	CCCGTTGGTTCAAGGGGTGGCGTGTTTTCATCGTGGGAATGACTCGACATCTTGGTTACCG
EM1600	CCTGCGCATCCGCGCAGGTTGGTGCAAGAGACAGGGTACGCAAGAGGGTCATTATATTTCG
EM1680	GCATCGGATCCCCGACCCGTACGAAAGGGTTCAACCTAAACAGTCCATATC
EM1771	TAATACGACTCACTATAGGGAGAGCAACCTTCGCCAGAATATTGCCA
EM1772	TACCATCGACACCGCCGACAATAA
EM1989	AATGATACGCATTATCTCAAGAGCAAATTCTGTCACTTCTACTCGACATCTTGGTTACCG
EM1990	CCTTCGTGCGCATGTTCATCTTCGCGGCAATCGCCTTCGGCACAAGAGGGTCATTATATTTCG
EM2024	TCTGGGATGATCACCTGCATACACAATAAGTCCACCGCGATGCTG
EM2025	CAGCATCGCGGTGGACTTATTGTGTATGCAGGTGATCATCCCAGA

EM2051	GAAGATGTGAGCGATAACCCATTGGATTTTCGTAGTTACCTCATGGAG
EM2052	CTCCATGAGGTAACTACGAAAATCCAATGGGTTATCGCTCACATCTTC
EM2065	TGTAATACGACTCACTATAGGATAACAAGAGCGTAACGATG
EM2066	CTGCGTACTGAATGATGA
EM2170	GCATCGGATCC CCGACCCGTA CGAAAGGGTT CAACCTAAAC ATTCCATATC TCCATGAGGTAACTACGAAAATCCAATGGGTTATCGCTCACATC
EM2171	GCATCGGATCCCCGACCCGTACGAAAGGGTTCAACCGGAACATTCCATATCTCCATGAGGT AAC
EM2173	TGTAATACGACTCACTATAGGGCTACATGGAGATTAAC
EM2174	CACGTCGTTGCTCAGCT

Supplementary References

Apirion D, Lassar AB (1978) A conditional lethal mutant of Escherichia coli which affects the processing of ribosomal RNA. *J Biol Chem* 253: 1738–1742.

Brickman TJ, Armstrong SK (1996) Colicins B and Ia as novel counterselective agents in interspecies conjugal DNA transfers from colicin-sensitive Escherichia coli donors to other gram negative recipient species. *Gene* 178: 39–42.

Cherepanov PP, Wackernagel W (1995) Gene disruption in Escherichia coli: TcR and KmR cassettes with the option of Flp catalyzed excision of the antibiotic-resistance determinant. *Gene* 158: 9–14.

Majdalani N, Cunning C, Sledjeski D, Elliott T, Gottesman S (1998) DsrA RNA regulates translation of RpoS message by an anti-antisense mechanism, independent of its action as an anti-silencer of transcription. *Proc Natl Acad Sci USA* 95: 12462–12467.

Mount DW (1977) A mutant of Escherichia coli showing constitutive expression of the lysogenic induction and error-prone DNA repair pathways. *Proc Natl Acad Sci USA* 74: 300–304.

Simons RW, Houman F, Kleckner N (1987) Improved single and multicopy lac-based cloning vectors for protein and operon fusions. *Gene* 53: 85–96.